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Aspicilia lixianensis, A. nivalis, and *A. pycnocarpa* spp. nov. from China

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Abstract — As a result of our study of *Aspicilia* from China, three new lichen species—*A. lixianensis, A. nivalis,* and *A. pycnocarpa*—are proposed and typified. Detailed descriptions, comparisons with related species, morphological photographs, and phylogenetic analysis of these taxa are provided.

Key words — ITS, *Megasporaceae, Pertusariales,* secondary metabolites, taxonomy

Introduction

Aspicilia A. Massal. is a large genus containing ca. 200 species (Lücking & al. 2016). Since being established by Massalongo in 1852, its taxonomic status has been very vague. It was long treated as a section of *Lecanora* Ach. (*Lecanoraceae*) for a long time (Magnusson 1940, 1952; Zahlbruckner 1930), until Hafellner (1984) restored *Aspicilia* to genus rank. Based on their three-gene phylogenetic analysis, Lumbsch & al. (2007) placed *Aspicilia* in *Megasporaceae.*

The main characteristics of *Aspicilia* are thallus crustose, rimose to areolate, prothallus present or not (if present, sometimes peripherally radiate or zonate); apothecia lecanorine, usually immersed, disk usually concave; epihymenium olive-green or dark brown and turning green with HNO_3; paraphyses usually moniliform or submoniliform; 8-spored asci;

TABLE 1. ITS sequences used in the phylogenetic analysis.
Newly generated sequences are in bold.

SPECIES	GENBANK	SPECIES	GENBANK
Aspicilia blastidiata	KX129963	*Aspicilia rivulicola*	EU057922
Aspicilia cinerea	MN906262	*Aspicilia subepiglypta*	KY249608
Aspicilia dendroplaca	HQ259260	*Aspicilia subgeographica*	KY249612
Aspicilia dudinensis	EU057906	*Aspicilia subgoettweigensis*	KY249614
Aspicilia goettweigensis	KX159293	*Aspicilia supertegens*	EU057938
Aspicilia lixianensis	**MW174005**	*Aspicilia virginea*	HQ259271
Aspicilia mashiginensis	EU057912	*Aspicilia volcanica*	NR158307
Aspicilia nivalis	**MW174002**		
Aspicilia permutata	EU057920	*Lobothallia crassimarginata*	JX476026
Aspicilia pycnocarpa	**MW174004**	*Lobothallia pruinosa*	JX476028

simple, colorless, and often ellipsoid ascospores (Owe-Larsson & al. 2007). *Aspicilia* species, which grow primarily on siliceous or calcareous rocks, are widely distributed throughout the world.

Forty species of *Aspicilia* have been reported in China (Hou & al. 2014, Ismayil & al. 2015, Li & al. 2013, Magnusson 1940, Shavkat & al. 2019, Zahlbruckner 1930), but many specimens in Chinese herbaria remain unidentified. After examining several specimen loans, we identified three *Aspicilia* species new to science, described in this paper.

Materials & methods

The specimens examined were deposited in Herbarium Mycologicum Academiae Sciences-Lichenes, Chinese Academy of Sciences, Beijing, China (HMAS-L) and the Plant Herbarium of Shandong Normal University, Jinan, China (SDNU).

DNA extraction, PCR amplification, sequencing

DNA was extracted from the specimens using CTAB method. The isolated DNA was amplified using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White & al. 1990). Each 25 μL sample contained 5.5 μL distilled H_2O, 5 μL DNA dilution, 1 μL of each primer, and 12.5 μL CWBio 0690 2xEs Taq MasterMix (Dye). The PCR mixture underwent a 5-minute initial denaturation at 95 °C, five cycles comprising 30 seconds at 95 °C + 30 seconds at 58 °C + 1 minute at 72 °C followed by 30–35 cycles with the annealing temperature decreased from 58 to 56 °C and concluded with final 7-minute extension at 72 °C (Sohrabi & al. 2013). PCR products were sequenced by BioSune Biotech Co. Ltd. in Beijing.

Phylogenetic analysis

We aligned three newly obtained ITS sequences with 14 ITS sequences belonging to *Aspicilia* and two *Lobothallia* sequences as the outgroup downloaded from GenBank (Table 1) using ClustalW in MEGA7 and optimized manually. A phylogeny was inferred by the Maximum Likelihood (ML) method based on the General Time Reversible model and Gamma Distributed in MEGA7 (Kumar & al. 2016).

Morphology and chemistry

We examined the morphology using a stereo microscope and a light microscope. The lichen products were detected by color reaction tests (CT) and thin-layer chromatography (TLC) using solvent systems A, B, and C (Elix 2014). The CT

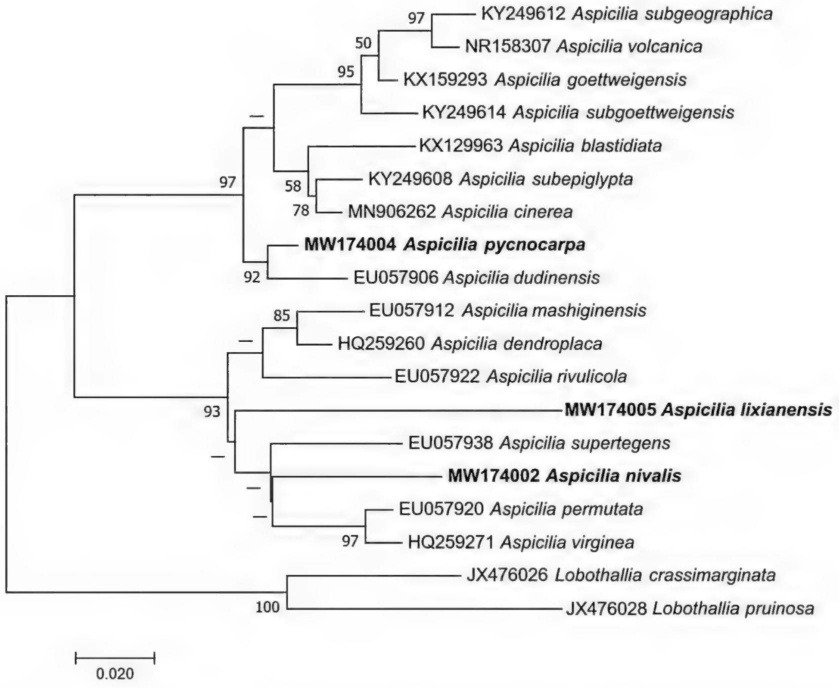
97
KY249612 *Aspicilia subgeographica*
50
NR158307 *Aspicilia volcanica*
95
KX159293 *Aspicilia goettweigensis*
–
KY249614 *Aspicilia subgoettweigensis*
KX129963 *Aspicilia blastidiata*
97
58
KY249608 *Aspicilia subepiglypta*
78
MN906262 *Aspicilia cinerea*
MW174004 *Aspicilia pycnocarpa*
92
EU057906 *Aspicilia dudinensis*
85
EU057912 *Aspicilia mashiginensis*
–
HQ259260 *Aspicilia dendroplaca*
EU057922 *Aspicilia rivulicola*
93
MW174005 *Aspicilia lixianensis*
–
EU057938 *Aspicilia supertegens*
–
MW174002 *Aspicilia nivalis*
–
EU057920 *Aspicilia permutata*
97
HQ259271 *Aspicilia virginea*
JX476026 *Lobothallia crassimarginata*
100
JX476028 *Lobothallia pruinosa*
0.020

Fig. 1. The most parsimonious ML tree generated from ITS sequences showing the phylogenetic placement of *Aspicilia pycnocarpa, A. lixianensis,* and *A. nivalis* (in bold) among 17 *Aspicilia* spp. and two *Lobothallia* spp. (outgroup). Bootstrap probabilities (50–100%) are indicated at internal branches.

reagents used were K (10% KOH in water), N (50% nitric acid), and IKI (Lugol's iodine solution).

ITS sequence analysis

Phylogenetic analysis produced a parsimonious tree with 50–100% bootstrap support values (FIG. 1). All *Aspicilia* species clustered within one monophyletic clade with the ITS sequence analysis supporting *Aspicilia pycnocarpa*, *A. lixianensis*, and *A. nivalis* as separate taxa. *Aspicilia pycnocarpa* appears to be closely related to *Aspicilia dudinensis*. *Aspicilia lixianensis* and *A. nivalis* are on separate branches, and no close relationships are indicated by the analysis.

Taxonomy

Aspicilia pycnocarpa Q. Ren & Lin Liu, **sp. nov.** FIG. 2

FN 570766

Differs from *Aspicilia cinerea* by its longer conidia and possession of stictic acid as its major lichen product.

TYPE: China, Jilin Province, route from Changbai County to Tianchi south slope scenic area, alt. 1300 m, on siliceous rock, 25 Jul. 2014, L. Hu 20141380 (**Holotype**, SDNU; GenBank MW174004).

ETYMOLOGY: from the Greek elements *pycno-* (crowded) + *-carpa* (fruit), referring to its abundant apothecia.

THALLUS crustose, areolate to rimose, 2.5–4.5 cm diam., 0.1–0.3 mm thick, pale brown to brown; areoles irregular, 0.2–1 mm diam., flat, continuous, fissured, cracks deep; prothallus inconspicuous. Upper cortex 25–30 μm thick, epruinose. Algal layer 20–30(–37.5) μm thick.

APOTHECIA prominent, abundant, aspicilioid, 1(–4) per areole, 0.1–0.7 mm diam.; disc brown to black, flat to slightly concave, epruinose, or sometimes slightly pruinose; thalline margin inconspicuous. True exciple to 37.5–62.5 *μ*m wide in lateral portion. Epihymenium olive-green to olive-brown, K+ brown, N+ green; Hymenium hyaline, IKI+ blue, 87.5–112.5 μm high; paraphyses not separating in KOH, distinctly moniliform with 2–6 globular cells; hypothecium colorless, IKI+ blue, 50–75 μm thick, with algae below the hypothecium. Asci clavate, *Aspicilia*-type, 8-spored; ascospores hyaline, simple, ellipsoid, (12.5–)17.5–21.25 × (8.75–)10–12.5 μm. Pycnidia black, round-shaped; conidia filiform, straight or slightly curved, (13.75–)17.5–25(–27.5) μm long.

SPOT TESTS—Upper cortex K+ yellow, sometimes turning red or orange; medulla K+ yellow.

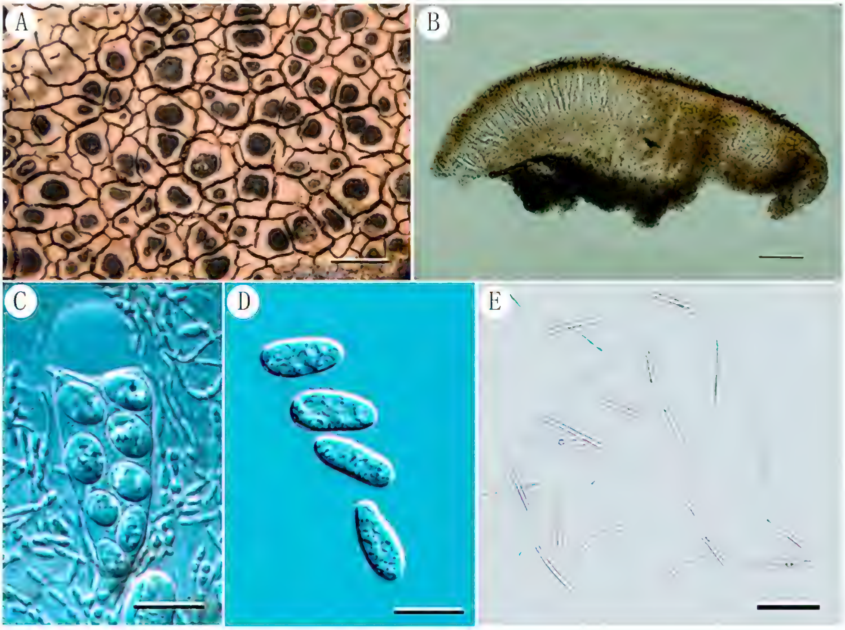

FIG. 2. *Aspicilia pycnocarpa* (holotype, SDNU 20141380). A. Thallus with apothecia; B. Apothecial section; C. Ascus with 8 ascospores; D. Ascospores: colorless, simple, ellipsoid; E. Filiform conidia. Scale bars: A = 1 mm; B = 100 µm; C–E = 20 µm.

SECONDARY METABOLITES—Stictic acid (major), norstictic acid (minor) and substictic acid (minor) (TLC).

ECOLOGY—On siliceous rock, interspersed with other lichens, such as *Lecidea* sp. Known only from the type locality.

> ADDITIONAL SPECIMEN EXAMINED—CHINA. JILIN PROVINCE: route from Changbai County to Tianchi south slope scenic area, alt. 1300 m, on siliceous rock, 25 Jul. 2014, L. Hu 20141379 (SDNU).

COMMENTS—*Aspicilia pycnocarpa* is characterized by its thin flat thallus, flat to slightly elevated thalline margin, abundant apothecia, medium ascospores, and long conidia. The specimens in SDNU were previously identified as *A. cinerea* (L.) Körb. based on the presence of norstictic acid, but *A. cinerea* has shorter conidia (11–16 µm) and contains mainly norstictic acid (Owe-Larsson & al. 2007). *Aspicilia pycnocarpa* is similar to *A. pacifica* Owe-Larss. & A. Nordin, which is distinguished by its higher hymenium (130–220 µm), larger ascospores (14–33 × 10–20 µm), and shorter conidia (8–17 µm) (Owe-Larsson & al. 2007).

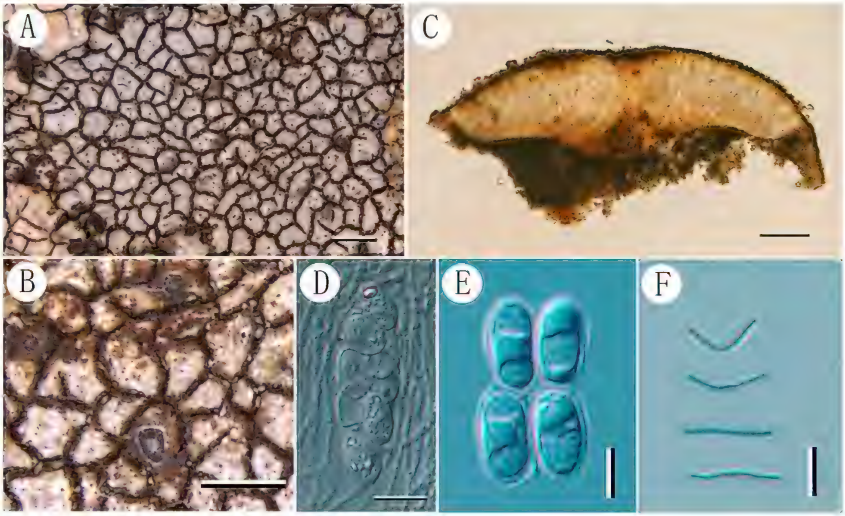

Fig. 3. *Aspicilia lixianensis* (holotype, HMAS-L 0139004). A. Thallus in moist environment; B. Thallus with apothecia; C. Apothecial section; D. Ascus with 8 ascospores; E. Ascospores: colorless, simple, ellipsoid; F. Filiform conidia. Scale bars: A = 2 mm; B = 0.5 mm; C = 100 µm; D = 20 µm; E, F = 10 µm.

In the ML-tree, *A. pycnocarpa* appears closely related to *A. dudinensis* (H. Magn.) Oxner, which differs by its usually disjointed pale or dark gray areoles, smaller ascospores (15–18 × 8.5–10 µm), and 18–22 µm long conidia (Magnusson 1952, as *Lecanora dudinensis*).

Aspicilia lixianensis Q. Ren & Lin Liu, **sp. nov.** Fig. 3

FN 570763

Differs from *Aspicilia asiatica* by its higher hymenium, from *Aspicilia subconfluens* by its lower hypothecium, and from both by its siliceous substrate.

Type: China, Sichuan Province, Lixian County, roadside of Jiabigou, 31.621 31.6214°N 1028455°E, alt. 2950 m, on siliceous rock, 10 Aug. 2016, W.C. Wang, WWC-51 (**Holotype**, HMAS-L 0139004; GenBank MW174005).

Etymology: referring to its type locality, Lixian County in Sichuan Province.

Thallus crustose, areolate to rimose, 0.5–1.5 cm diam., ±0.3 mm thick, gray to dark gray; areoles irregular, 0.2–1 mm diam., flat or slightly convex, continuous, fissured, cracks deep; prothallus inconspicuous. Upper cortex 12.5–25 µm thick, densely pruinose. Algal layer 37.5–50 µm thick.

Apothecia prominent, aspicilioid, usually solitary in the areoles, 0.25–0.7 mm diam.; disc black, flat to slightly convex, with dense white pruina in center; thalline margin usually inconspicuous. True exciple inconspicuous. Epihymenium olive-brown, K+ brown, N+ green; hymenium hyaline, IKI+ blue, 125–167.5 µm high; paraphyses separating in KOH, distinctly moniliform with 4–6 globular cells; hypothecium colorless, IKI+ blue, 37.5–87.5 µm thick, with algae below the hypothecium. Asci clavate, *Aspicilia*-type, 8-spored; ascospores hyaline, simple, ellipsoid, (10–)12.5–17.5(–20) × (6.25–)8.75–12.5 µm. Pycnidia black, stellate-shaped; conidia filiform, straight or curved, (10–)15–20 µm long.

Spot tests—Upper cortex K+ pale yellow; medulla K+ yellow.

Secondary metabolites—Stictic acid (major), substictic acid (minor) (TLC).

Ecology—On siliceous rock, growing with *A. cinerea*. Known only from the type locality.

Comments—*Aspicilia lixianensis* is characterized by its dark gray upper surface with white pruina, black disks covered by densely white pruina, medium ascospores and conidia, and the presence of stictic and substictic acids. This specimen had few apothecia and abundant pycnidia and was surrounded by *A. cinerea*.

Morphologically, *A. lixianensis* resembles *A. asiatica* (H. Magn.) Yoshim. and *A. subconfluens* (H. Magn.) J.C. Wei, but those two species grow on slightly calciferous rocks in arid and semi-arid areas. Additionally, *A. asiatica* has lower hymenium (85–100 µm) (Magnusson 1940, as *Lecanora asiatica*) while the hypothecium of *A. subconfluens* is usually higher (150–200 µm; Magnusson 1940, as *Lecanora subconfluens*).

Aspicilia nivalis Q. Ren & Lin Liu, **sp. nov.** Fig. 4

FN 570764

Differs from *Aspicilia decorticata* by its white thallus, its higher hymenium, and its larger ascospores.

Type: China, Xizang, Jiangda County, Xuejila Mountain, 31.445°N 98.0547E, alt. 4300 m, on sandstone, 10 Aug. 2016, W.C. Wang, WWC-148 (**Holotype**, HMAS-L 0139012; GenBank MW174002).

Etymology: from *nivalis,* referring to the rough snow-white thallus and high elevation snow-covered (in winter) type locality on Xuejila Mountain.

Thallus crustose, areolate, 1–3.5 cm diam., ±0.5 mm thick, white; areoles irregular, 0.5–1.5 mm diam., flat, continuous, rough, slightly to deeply fissured; prothallus inconspicuous. Upper cortex 25–50 µm thick, powdery. Algal layer 50–62.5 µm thick.

Apothecia prominent, aspicilioid, 1(–3) per areole, 0.2–0.6 mm diam.; disc black, slightly concave, with dense white pruina; thalline margin conspicuous and flat, usually forming a black rim. True exciple inconspicuous. Epihymenium dark brown, K+ brown, N+ green; hymenium hyaline, IKI+ blue, 125–150 µm high; paraphyses separating in KOH, distinctly moniliform with 4–6 globular cells; hypothecium colorless, IKI+ blue, 25–45 µm thick, without algae below the hypothecium. Asci clavate, *Aspicilia*-type, 8-spored; ascospores hyaline, simple, ellipsoid, 18.75–30 × 10–15 µm. Pycnidia black, round-shaped; conidia filiform, straight or curved, 17.5–25 µm long.

Spot tests—Upper cortex K–; medulla K–.

Secondary metabolite—Substictic acid (TLC).

Ecology—On red-brown sandstone. Known only from the type locality.

Comments—*Aspicilia nivalis* is characterized by its continuous and extremely rough thallus with powdery surface, large ascospores, and long conidia. It is similar to *A. decorticata* (H. Magn.) J.C. Wei, which differs by having a pale yellowish thallus, lower hymenium (85–100 µm), smaller ascospores (12–15 × 7.5–8.5 µm), and calciferous rock substrate (Magnusson 1940, as *Lecanora decorticata*). The new species is also similar to *A. persica* (Müll. Arg.) Sohrabi, which differs by growth on siliceous rock, densely pruinose upper cortex, shorter ascospores (17.5–22.5 µm), and different secondary metabolites (stictic acid, ±norstictic acid) (Li & al. 2013). *Aspicilia nivalis* also resembles *A. albocretacea* (Zahlbr.) J.C. Wei, separated by its continuous thallus with few cracks, epruinose discs, and shorter ascospores (19–23 µm; Zahlbruckner 1930, as *Lecanora albocretacea*).

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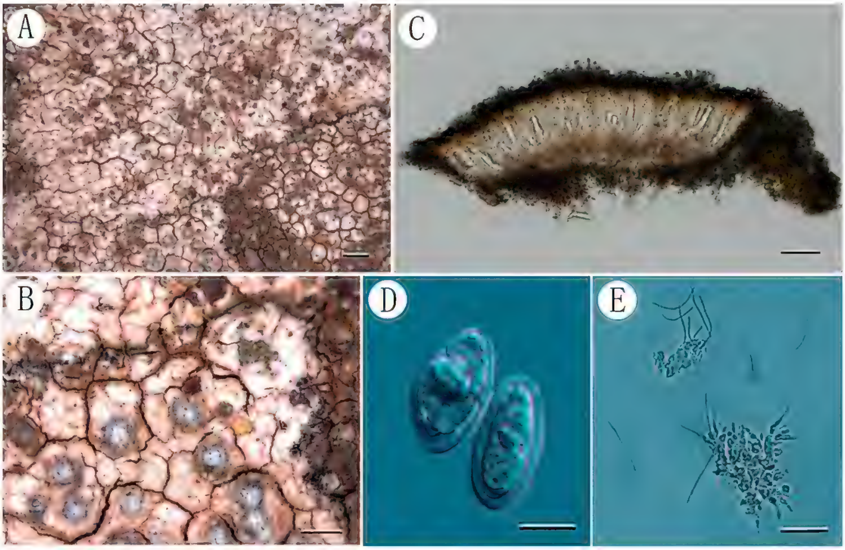

FIG. 4. *Aspicilia nivalis* (holotype, HMAS-L 0139012). A. Thallus on sandstone; B. Thallus with apothecia; C. Apothecial section; D. Ascospores: colorless, simple, ellipsoid; E. Filiform conidia. Scale bars: A = 1 mm; B = 0.5 mm; C = 100 µm; D = 10 µm; E = 20 µm.

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